Datasheet for ABIN612781

KIT Ligand ELISA Kit

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Overview

Quantity:	96 tests
Target:	KIT Ligand (KITLG)
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	0.02 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human SCF ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human SCF in plasma, serum and cell culture supernatant
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Human SCF Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human SCF. 1 Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Human SCF Standard: Human SCF in a buffered protein base (160 ng, lyophilized). Biotinylated SCF Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against SCF (80µl). EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).

Product Details

	Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80µl). Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).
Material not included:	Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water

Target Details

Target:	KIT Ligand (KITLG)
Alternative Name:	Stem Cell Factor (SCF) (KITLG Products)
Background:	Stem Cell Factor (SCF) is known as c-Kit receptor ligand, KL, steel factor, or mast cell growth factor and is expressed in fibroblasts, thymus tissue, spleen, testes, placenta and mast cells. SCF is a cytokine that exists in two forms produced by alternative splicing: a soluble form of approximately 31 kDa and a membrane-bound form of approximately 32 kDa, lacking the proteolytic site for processing into the soluble form (1-4). SCF not only plays an important role in hematopoiesis, reproduction, melanogenesis and tumor progression, but also involved in proliferation and differentiation of mast cells. It stimulates mast cell activation in human bronchi and induces smooth muscle cell contraction. Both increased expression of SCF and its receptor c-Kit were found in asthma patients. During chronic stroke, SCF in combination with granulocyte-colony stimulating factor (G-CSF) treatment can enhance repair of brain damage. Blocking SCF-c-kit signaling is sufficient to inhibit lung cancer stem cell proliferation and survival promoted by chemotherapy.
Pathways:	RTK Signaling, Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Neurotrophin Signaling Pathway

Application Details

Sample Volume:	50 µL
Assay Time:	< 4 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures human SCF in less than 4 hours. A polyclonal antibody specific for human SCF has been pre-coated onto a 96-well microplate with removable strips. SCF in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for

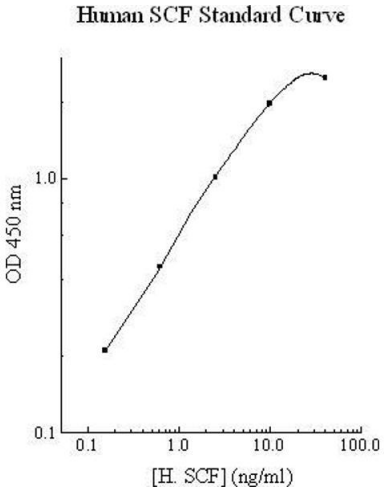
	SCF, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. EIA Diluent Concentrate (10x): Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 160 ng of SCF Standard with 4 ml of EIA Diluent to generate a standard solution of 40 ng/ml. Allow the standard to sit for 10 minutes with 2 gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (40 ng/ml) 1:4 with EIA Diluent to produce 10, 2.5, 0.63, and 0.156 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [SCF] (ng/ml) P1 Standard (40 ng/ml) 40.00 P2 1 part P1 + 3 part EIA Diluent 10.00 P3 1 part P2 + 3 part EIA Diluent 2.500 P4 1 part P3 + 3 part EIA Diluent 0.625 P5 1 part P4 + 3 part EIA Diluent 0.156 P6 EIA Diluent 0.000 Biotin SCF Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): Dilute the Wash Buffer Concentrate 1:20 with reagent grade water. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:2 into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:2 into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.
Assay Procedure:	Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20 - 30 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 µL of SCF standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition. Wash

Application Details

	five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. Add 50 µL of Biotinylated SCF Antibody to each well and incubate for one hour. Wash a microplate as described above. Add 50 µL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash a microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for about 15 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings. 3
Calculation of Results:	Calculate the mean value of the triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.5% and 7.2% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store kit at 2-8°C or -20°C upon arrival up to the expiration date. Opened EIA Diluent may be stored for up to 1 month at 2-8°C. Store reconstituted reagents at -20°C or below. Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.



ELISA

Image 1.